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Short Communication

Comparison of the effects of thermal stress and CO_2-driven acidified seawater on fertilization in coral *Acropora digitifera*

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Summary

Global warming (GW) and ocean acidification (OA) have been recognized as severe threats for reef-building corals that support coral reef ecosystems, but these effects on the early life history stage of corals are relatively unknown compared with the effects on calcification of adult corals. In this study, we evaluated the effects of thermal stress and CO_2-driven acidified seawater on fertilization in a reef-building coral, *Acropora digitifera.* The fertilization rates of *A. digitifera* decreased in response to thermal stress compared with those under normal seawater conditions. In contrast, the changes of fertilization rates were not evident in the acidified seawater. Generalized Linear Mixed Model (GLMM) predicted that sperm/egg crosses and temperature were explanatory variables in the best-fitted model for the fertilization data. In the best model, interactions between thermal stress and acidified seawater on the fertilization rates were not selected. Our results suggested that coral fertilization is more sensitive to future GW than OA. Taking into consideration the previous finding that sperm motility of *A. digitifera* was decreased by acidified seawater, the decrease in coral cover followed by that of sperm concentration might cause the interacting effects of GW and OA on coral fertilization.

Keywords: Coral, Fertilization, Global warming, Interaction, Ocean acidification

Introduction

The efficiency of fertilization is crucial to the survival of sessile species of marine invertebrates, especially in species that release gametes into the water column. In such cases, sperm concentrations are diluted rapidly, reducing the frequency of sperm–egg collisions (Levitan & Petersen, 1995). In the case of sessile marine invertebrates, such as corals, efficiency of fertilization is critical for the maintenance of their life cycle. *Acropora* is one of the most widespread, abundant, and species-rich (113–180 species) genera of coral (Wallace, 1999; Veron, 2000). *Acropora* species release their gametes as buoyant bundles into the water column, and fertilization occurs at the sea surface. This situation suggests that a fertilization event of *Acropora* species would be easily affected by environmental changes.

Global warming (GW) and ocean acidification (OA) caused by increased atmospheric CO_2 partial pressure (pCO_2) through human activities are environmental problems of high concern at present (Hoegh-Guldberg *et al.*, 2007). Current estimates predict that the temperature would increase ~4°C and pCO_2 could reach around 1000 μatm by the end of this century (IPCC, 2007). Reef-building corals are known to be sensitive to such environmental changes by GW and OA. In particular, increased temperature is considered to be a key driver of coral bleaching, which results in

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Table 1 Summary of physical and chemical conditions in each treatment

Conditions	Temperature (°C)	pH_T	pCO_2 (μatm)	HCO_3^-	CO_3^{2-}	Ω_{arg}
a	27.2 ± 0.1	7.99 ± 0.02	438 ± 30	1683 ± 20	189 ± 8	3.04 ± 0.13
b	27.0 ± 0.1	7.68 ± 0.02	990 ± 52	1893 ± 11	105 ± 4	1.68 ± 0.07
c	31.3 ± 0.1	7.94 ± 0.02	500 ± 29	1673 ± 17	193 ± 7	3.18 ± 0.11
d	31.6 ± 0.4	7.64 ± 0.02	1111 ± 64	1878 ± 13	111 ± 5	1.83 ± 0.09

Mean values and standard deviations are shown for each parameter. (a) normal temperature and pCO_2, (b) normal temperature and high pCO_2, (c) high temperature and normal pCO_2, (d) high temperature and pCO_2.

the collapse of the association between reef-building corals and their symbiotic algae (zooxanthellae, genus *Symbiodinium*; Hoegh-Guldberg, 1999). Ocean acidification has been recently recognized as a new threat to corals because their calcification rates are generally reduced by the decrease in carbonate ion concentrations (Kleypas *et al.*, 2006; Hoegh-Guldberg *et al.*, 2007).

Early life history stages of corals have been recently reported to be sensitive to thermal stress and acidified seawater (Negri *et al.*, 2007; Albright *et al.*, 2010; Morita *et al.*, 2010; Suwa *et al.*, 2010; Albright & Mason, 2013; Chua *et al.*, 2013). However, reports on the interacting effects of thermal stress and acidified seawater on the early life history stages of corals are still limited (Albright & Mason, 2013; Chua *et al.*, 2013). In this study, by assuming future environmental changes, we tried to evaluate the effects of thermal stress and CO_2-driven acidified seawater on fertilization of a reef-building coral, *Acropora digitifera*, which is one of the dominant species around the Ryukyu Archipelago, Okinawa, Japan (Nakajima *et al.*, 2010).

Materials and methods

Four seawater conditions (normal temperature and pCO_2, normal temperature and high pCO_2, high temperature and normal pCO_2, and high temperature and pCO_2) were prepared in four aquaria (12 l). The seawater temperature was maintained with a thermostat and a heater. Precise and stable pCO_2 conditions (each pCO_2 value was maintained within a 10% fluctuation during the experimental period) were achieved by using a pCO_2 control system called the Acidification Impact on CALcifiers (AICAL) system (Fujita *et al.*, 2011), which monitors pCO_2 using a non-dispersive infrared absorption (NDIR) system with a LI-COR 840 detector (LI-COR Biosciences Co., Lincoln, NE, USA). Seawater was filtered using an inline filter system (1 μm). The chemical and physical conditions of each treatment are summarized in Table 1. The pH, HCO_3^-, CO_3^{2-}, Ω_{arg} were estimated from pCO_2, temperature, mean total alkalinity of 2152 ± 86 μmol/kg (mean ± standard deviation), and salinity of 34.5 using the computer program CO2SYS (Lewis & Wallace, 1998).

Gravid coral colonies were collected from a fringing reef at Sesoko Island, Japan in May 2009. All samples were collected in strict accordance with good animal practice as defined by the relevant national and/or local animal welfare bodies, and all sampling that required permission for this study within Okinawa Prefecture was approved by the prefecture. Gametes were collected and prepared in accordance with Morita *et al.* (2006). Five sperm/egg crosses using five colonies of *A. digitifera* were performed (sperm from one colony and eggs from another one for each cross). For these crosses, five colonies were used for sperm preparation, and eggs were obtained from four colonies. All crosses were performed in 10 ml volumes (20 ml vial) and replicated three times in each cross. Four egg batches without addition of sperm were also prepared using eggs from four colonies as negative controls (three replicates). The lids of vials that contained seawater adjusted to treatment values were firmly closed and vials were floated in each aquarium. Between 30 and 80 eggs were incubated for 15 min in vials that contained adjusted seawater before sperm were added. An optimal concentration of 10^5 sperm/ml (Willis *et al.*, 1997) was used for each cross. Finally, 30 min after addition of sperm, sperm were removed to avoid the excess of fertilization in accordance with Iguchi *et al.* (2009). This process would also help to reduce the effect of change of pCO_2 in vials on fertilization. Fertilized eggs were fixed with 3–4% formalin 6 h after addition of sperm, and the numbers of unfertilized eggs and developing embryos were counted under a dissecting microscope.

We applied the Generalized Linear Model (GLM) fitted with a binomial error distribution and logit link function to analyze fertilization data (explanatory variables: crosses, temperature, pCO_2, temperature × pCO_2). However, over-dispersion was observed in the GLM analysis (data not shown), thus, the Generalized Linear Mixed Model (GLMM) fitted with a binomial error distribution and logit link function was applied with the same responsive variables as above, but based

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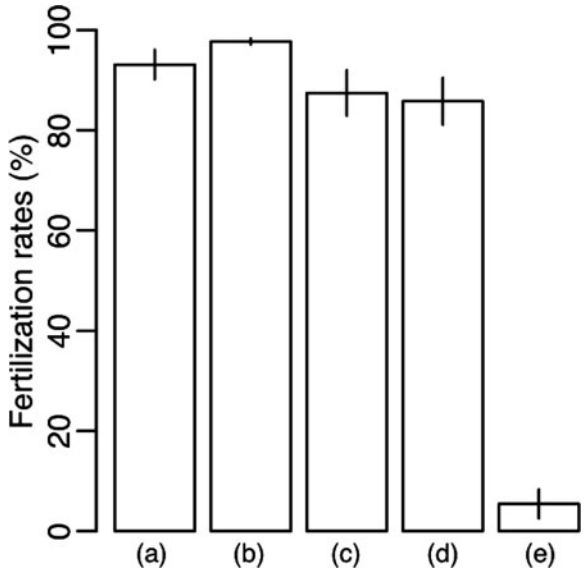

Figure 1 Fertilization rates (%) of *Acropora digitifera* eggs in each treatment. Bars show average fertilization rates and standard errors for five crosses ($n = 15$) and four egg batches ($n = 12$). Each treatment was repeated three times. Sperm were added at a concentration of 10^5 sperm/ml. (a) Normal temperature and pCO_2. (b) Normal temperature and high pCO_2. (c) High temperature and normal pCO_2. (d) High temperature and pCO_2. (e) Eggs without the addition of sperm.

on an Akaike information criteria (AIC; Burnham & Anderson, 2002). These statistical analyses were performed using R (R Development Core Team, 2011).

Results and Discussion

In our fertilization trial, high fertilization rates of *A. digitifera* were observed in all treatments (average 92.1 %; Figure 1). The low fertilization rates detected in some self crosses were probably due to low levels of cross-contamination that occurred during removal of sperm from each treatment. In the statistical analysis, we applied GLM for fertilization data, but over-dispersion was observed. To overcome this problem, we applied GLMM and temperature and crosses were selected in the best-fitted model (Table 2). In a previous study, we found some variations among crosses in coral fertilization (Iguchi *et al.*, 2009), thus we incorporated crosses in our model. But considering that our GLM analysis that included crosses as an explanatory variable was not enough for avoiding over-dispersion, variations among fertilization replicates within a cross could not be ignored.

Decreases in fertilization rates were observed at high temperatures conditions of both non-acidified and acidified seawater in comparison with that at the normal temperature control. This finding is consistent with the previous finding for the fertilization rates of *A. millepora*, which also decreased under thermal stress (Negri *et al.*, 2007). However, the decrease in fertilization rates was not evident between non-acidified and acidified seawater. The interaction between thermal stress and acidified seawater on fertilization was also unclear. These results were also supported by the statistical analysis using GLMM of which best-fitted model incorporated only crosses and temperature as explanatory variables.

Contrary to the previous finding that reduced flagellar motility was observed in *A. digitifera* even with only a slight decrease of pH (Morita *et al.*, 2010), we could not detect a decrease in fertilization rates for *A. digitifera* in acidified seawater. We tried to avoid the excess fertilization by removal of sperm in accordance with the method by Iguchi *et al.* (2009), but a decrease in sperm binding of 99.99% may still allow fertilization to occur (Iguchi *et al.*, 2007). The reason why we could not detect decreases in fertilization rates in acidified seawater could be attributed to our experimental conditions that used only a single sperm

Table 2 The top-ranked candidate models for each explanatory variable

Model	Deviance	AIC	ΔAIC	AICW
Crosses + temperature	138.3	152.3	0.00	0.458
Crosses + temperature + pCO_2 + pCO_2 × temperature	135.1	153.1	0.82	0.304
Crosses + temperature + pCO_2	137.8	153.8	1.54	0.212
Crosses	146.7	158.7	6.40	0.019
Crosses + pCO_2	146.4	160.4	8.16	0.008
Temperature	165.5	171.5	19.17	0.000
Temperature + pCO_2 + pCO_2 × temperature	165.1	173.2	20.96	0.000
Null	171.1	175.1	22.78	0.000
pCO_2	170.9	176.9	24.61	0.000
Temperature + pCO_2	169.2	177.2	24.90	0.000

Model deviance, AIC, difference in AIC from the best-fitted model (ΔAIC) and weight (AICW) values are given for each model.

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concentration (10^5 sperm/ml), because previously researchers have reported decreases in fertilization rates in acidified seawater using several lower sperm concentrations (Albright *et al.*, 2010; Albright & Mason, 2013). Although the sperm concentrations used in our study were high, in order to detect the effect of OA on fertilization rates, it seems likely that fertilization of *A. digitifera* is more sensitive to future GW than to OA.

In the field, high sperm concentrations to enable sufficient fertilization rates are also observed during coral mass spawning (Willis *et al.*, 1997; Omori *et al.*, 2001), but the decrease in coral cover followed by that of sperm concentration might cause interacting negative effects of GW and OA on the fertilization of *A. digitifera*, as Albright & Mason (2013) reported interacting negative effects of GW and OA on the fertilization of *Acropora tenuis* at low sperm concentrations. To further understand the interacting effects of future GW and OA on the coral reef ecosystem, these effects on the early life history stages of corals should also be taken into account.

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